Effects of Cigarette Smoking on Metabolism and Effectiveness of Systemic Therapy for Lung Cancer

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Introduction: Cigarette smoke associated polycyclic aromatic hydrocarbons can induce key drug-metabolizing enzymes of cytochrome P450 and isoforms of the glucuronyl transferases families. These enzymes metabolize several systemic therapies for lung cancer. Induction of these enzymes may lead to accelerated clearance with resultant impact on systemic therapy efficacy and toxicity in smokers compared with nonsmokers. This article reviews published literature regarding the influence of smoking as it relates to alteration of metabolism of systemic therapy in lung cancer.

Methods: A structured search of the National Library of Medicine's PubMed/MEDLINE identified relevant articles. Data were abstracted and analyzed to summarize the findings.

Results: Studies that analyzed pharmacokinetic data were prospective. Smokers receiving erlotinib exhibited rapid clearance, requiring a higher dose to reach equivalent systemic exposure compared with nonsmokers. Smokers receiving irinotecan also demonstrated increased clearance and lower systemic exposure. There was no difference in clearance of paclitaxel or docetaxel in smokers. Chemotherapy-associated neutropenia was worse in nonsmokers compared with smokers in patients treated with paclitaxel, docetaxel, irinotecan, and gemcitabine.

Conclusions: Systemic therapy for lung cancer has a narrow therapeutic index such that small changes in plasma concentrations or exposure in smokers may result in suboptimal therapy and poor outcomes. Smoking cessation must be emphasized at each clinical visit. However, prospective trials should take into consideration the effects of smoking history on drug pharmacokinetics and efficacy. The metabolizing enzyme phenotype in smokers may require individualized dose algorithms for specific agents.

Key Words: Pharmacokinetics, Pharmacodynamics, Smoking, Chemotherapy metabolism, Nicotine, Response, Toxicities, Lung cancer

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Cigarette smoking is a significant source of morbidity and mortality. According to the National Health Interview Survey, approximately one in five (20.2%) of US adults currently smoke.[1] In addition, there is an estimated 49.9 million former smokers in the United States.[2] Smoking is the greatest risk factor for lung cancer, which continues to be the leading cause of cancer-related death for both sexes.[3] Outcomes in lung cancer for both current and former smokers are dismal, with a 5-year relative survival rate of approximately 15%.[4] It is also evident that nonsmokers have an improved survival from therapy for advanced lung cancer compared with smokers.[4–6] The recent Surgeon General Report highlights 50 years of progress in tobacco control and prevention and presents new data on the health consequences of smoking. This may relate to a multitude of factors ranging from intrinsic differences in lung cancer biology, host associated medical comorbidities[7] and polymorphisms in drug-metabolizing enzymes[8] leading to reduced efficacy of therapies.

In addition to the carcinogenic effects of tobacco products, the components of cigarette smoke can induce drug-metabolizing enzymes, which have been demonstrated in both in vitro and animal models.[9–11] Induction of these metabolizing enzymes resulting in accelerated clearance may reduce drug efficacy in smokers and impact clinical outcomes. Smoking is associated with reduced beta blocker effectiveness, in terms of lowering blood pressure and heart rate, and reduced sedation from benzodiazepines.[12] Several often used chemotherapeutic drugs and many of the newer targeted therapies are metabolized by the hepatic cytochrome P450 enzymes, in addition to the uridine 5′-diphosphate-glucuronyl transferases. Although the exact mechanism behind the accelerated drug metabolism has not yet been clearly elucidated, there is emerging evidence that compounds in cigarette smoke may epigenetically modify these enzymes that result in persistently elevated activity, even after smoking cessation.[13] In addition, there may be a direct effect of nicotine on molecular effectors of cellular apoptosis induced by several chemotherapies for lung cancer.[14]

Several studies have reported the effects of cigarette smoking on the ,pharmacokinetic (PK) and pharmacodynamic (PD) effects of systemic for lung cancer. The goal of this article is to review the effects of cigarette smoke as it relates to metabolism and efficacy of systemic therapies for lung cancer. We searched PubMed/MEDLINE to identify relevant articles published between 2000 and January 2014. The search strategy included Medical Subject Headings (MeSH) and keywords representing the concepts of smoking status, smoking cessation or nicotine replacement combined with MeSH and keywords for lung cancer, chemotherapy, and selected

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chemotherapeutic agents (docetaxel, gemcitabine, etc.). Citations were filtered to exclude citations solely on "never smokers," and then filtered again using MeSH, subheadings, and keywords to identify articles that focused on molecular rather than metabolic or pharmacokinetic responses to chemotherapy. Data were abstracted from 171 publications and summarized below.

EFFECTS OF CIGARETTE SMOKE ON PHASE I AND II METABOLIZING ENZYMES

Cigarette smoke is known to contain more than 7000 chemicals, of which more than 60 possess carcinogenic properties.[2] Smoke is composed of both volatile and particulate phases that comprise approximately 95% and 5%, respectively. The volatile phase is composed primarily of nitrogen, oxygen, and carbon dioxide.[15] Excluding the alkaloid and the water content, the remaining particulate mass is referred to as tar, which is composed of carcinogens including polycyclic aromatic hydrocarbons (PAHs), *N*-nitrosamines, and aromatic amines.[16] PAHs are compounds with two or more aromatic and cyclic rings that can induce DNA mutations.[17,18] More than 500 PAHs have been identified in cigarette smoke.[19] PAHs are oxidized by cytochrome P450 enzymes and the resultant metabolites exert mutagenic effects on DNA. The same PAHs have also been shown to induce members of the P450 enzyme family, which normally process xenobiotics.[15,20,21] The P450 enzymes are responsible for phase I drug metabolism by oxidizing the parent compound to a more readily excreted metabolite.[22] The most common isoforms as relates to metabolism of often used systemic therapy for lung cancer include CYP1A1/2, CYP2D6, and CYP3A4. Herein, we will review how tobacco smoke interacts with these enzymes either through increased induction or increased degradation.

CYP1A1 and CYP1A2 are the most common CYP1 family isoforms that metabolize some systemic therapies used to treat lung cancer such as erlotinib, an epidermal growth factor (EGFR) inhibitor. PAHs in tobacco smoke can induce isoenzymes CYP1A1 and CYP1A2.[20] Zhu et al.[23] theorized that the process of selective induction of a CYP isoform by PAHs is primarily determined by the following three core elements: each inducible CYP isoform has a corresponding intracellular receptor that interacts with the inducer chemicals, each isoform and its receptor may share highly similar steric structures, and each inducible *CYP* gene may have a distinct genomic response element that interacts selectively with the corresponding receptor. The CYP1A1 enzyme, an aryl hydrocarbon hydroxylase, is involved in the activation of procarcinogens, such as PAHs, and can be transcriptionally induced by PAHs.[24,25] Binding to the aryl hydrocarbon receptor, a basic helix-loop-helix transcription factor, leads to heterodimerization and binding to the aryl hydrocarbon-responsive elements in the *CYP1A1* gene,[26] as detailed in Figure 1*A*. These events then lead to increased expression of the *CYP1A1* gene and potentially accelerated drug metabolism.[27,28] Anttila et al.[29] observed smoking-related alterations of the *CYP1A1* promoter methylation status in lung tumor samples. DNA from resected lung tumors demonstrated complete or partial

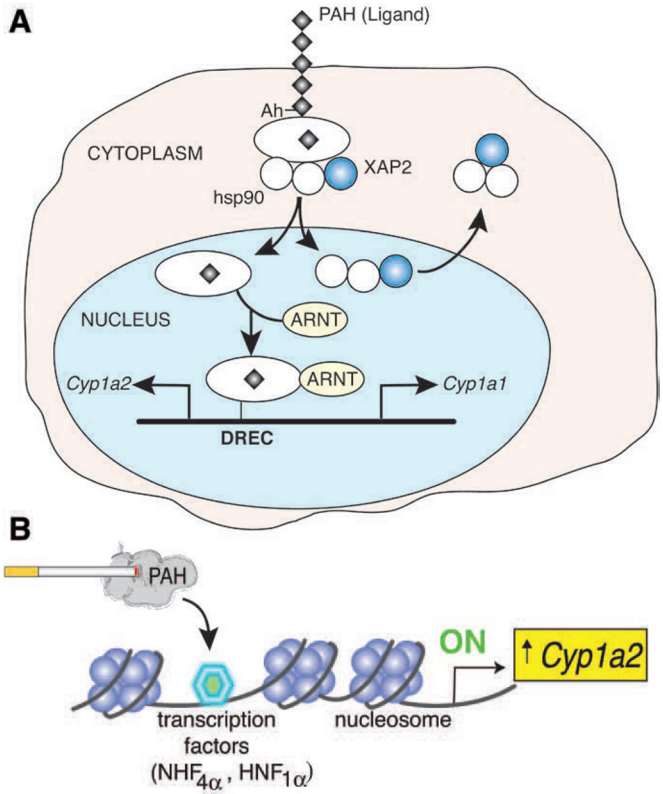

FIGURE 1. PAH-induced direct (*A*) transcriptional and (*B*) epigenetic regulation of cytochrome P450 enzymes, CYP1A1, and CYP1A2. PAH, polycyclic aromatic hydrocarbon; Ah, aryl hydrocarbon; ARNT, aryl hydrocarbon receptor nuclear translocator; Hsp90, heat shock protein 90; XAP2, X-associated protein.

CYP1A1 promoter methylation in 33% of heavy smokers, 71% of light smokers, and 98% of nonsmokers.[29]

Similarly, CYP1A2 is a hepatic enzyme that is responsible for the metabolism of several often used medications, such as theophylline, caffeine, and acetaminophen.[12] Induction of CYP1A2 may be mediated through binding of PAHs similar to CYP1A1, which leads to transcriptional activation of the *CYP1A2* gene.[30] Alternatively, as detailed in Figure 1*B*, PAH can epigenetically modify transcription factors such as $NHF_{4\alpha}$ and $HNF_{1\alpha}$, which leads to the upregulation of CYP1A2.[31,32] Cigarette smoke induces chromatin remodeling by acetylating lysine residues on histone proteins to facilitate gene expression.[33] In Addition, the activity of histone deacetylases, which remove acetyl groups to repress transcription were reduced activity in bronchial biopsies from smokers compared with nonsmokers ($p < 0.01$).[34] Induction of CYP1A2 is linked to increased activity of the enzyme which in turn leads to reduced serum concentrations and reduced efficacy of the substrates.[35]

CYP2D6, the most common isoform of the CYP2 family, is involved in metabolism of opiates used in supportive care for lung cancer patients. The gene encoding this enzyme has been reported to have multiple single nucleotide polymorphisms (SNPs) that can lead to varying expression among the

population. These different SNPS are associated with specific phenotypes namely extensive metabolizers, ultra rapid, and slow metabolizers. Approximately, 7.5% of Europeans and white Americans are slow metabolizers, whereas <2% of Asians and African Americans are slow metabolizers. Other isoforms such as CYP2E1, which biotransforms many compounds, including clinical drugs such as acetaminophen is also induced by nicotine leading to lower plasma concentrations of CYP2E1 substrates.[36]

CYP3A4 is the most abundant P450 isoform in the liver and gut and responsible for the metabolism of several often used drugs and systemic therapies for lung cancer, including the taxanes, gefitinib, and erlotinib. This increase in transcription leads to increased activity of the enzymes which in turn can affect PK of drugs metabolized by the CYP3A4 isoform. Examples include erlotinib as discussed below. PAHs are also known to induce some isoforms of the uridine diphosphate glucuronyltransferase family, which includes Phase II drug-metabolizing enzymes responsible for glucuronic acid conjugation.[12] Studies have shown increased glucuronidation rates of drugs such as codeine and propranolol in smokers.[37,38] Summarizing, cigarette smoke can lead to increased activation of several phase I and II drug-metabolizing enzymes. The increased activation may be related to direct transcriptional or epigenetic regulation by the PAHs present in smoke.

PK AND PD EFFECTS RELATED TO INDUCTION OF METABOLIZING ENZYMES

Induction of the cytochrome P450 enzymes and the glucuronyl transferases has a direct impact on both PK and PD parameters related to drugs metabolized by these enzymes. Some of the most clinically significant PK interactions between various nonchemotherapeutic drugs and smoking have been detailed in a review by Kroon,[39] who notes that caffeine, clozapine, olanzapine, and fluvoxamine all exhibit significant alterations to PK properties with cigarette smoke exposure, suggesting that smoking history must be taken into account when designing treatment plans. Similarly, smoking has been shown to cause PD drug interactions with hormonal contraceptives and inhaled corticosteroids.[40,41] Thus, although the exact mechanism of accelerated metabolism has not yet been clearly elucidated in all cases, it can be hypothesized that it may be because of both direct transcriptional effects of smoking on the CYP isoenzymes as described above or epigenetic effects on these enzymes, which keep them persistently activated for some time, even after smoking cessation.[42]

Short term we know that for the enzyme to be induced by cigarette smoke, additional amounts of the enzyme must be produced. Once the inducing agent has been discontinued, these enzymes continue to be induced because they do not simply cease to exist. Therefore, it is not until these enzymes have run their natural lifespan that we begin to see CYP metabolism return to normal.[43] In addition, new data emerging suggest that epigenetic effects of smoking may have a greater impact on sustained induction of CYP enzymes. Currently, there are no data to parse out whether cigarette smoke associated induction of P450 enzymes is transcriptional, epigenetic, or a combination thereof. Regardless, it has been posited that smokers prescribed certain medications that are substrates to the P450 enzymes may require a higher dose than nonsmokers and, conversely, may require dose reduction upon cessation of smoking.[12]

EFFECTS OF NICOTINE ON SYSTEMIC THERAPY FOR LUNG CANCER

Nicotine, an alkaloid, is considered to be the addictive component of tobacco. Cotinine, the main metabolite of nicotine, has been shown to be a reliable marker of nicotine exposure and more reflective of recent rather than acute nicotine use, which provides a better assessment of baseline nicotine exposure.[44] There are no prospective studies describing the effects of nicotine or nicotine derivatives on alteration of metabolism of systemic therapies for lung cancer. The literature, however, is rife with studies that demonstrate that nicotine affects various signaling pathways in lung cancer that are associated with resistance to chemotherapy used to treat lung cancer. Nicotine binds to high-affinity nicotine acetylcholine receptors (nAChRs) that are found on both normal and malignant human lung cells.[45–47] After binding to the nAChR, nicotine can stimulate multiple signaling pathways including the RAS pathway, leading to increased cellular proliferation. In addition, nicotine inhibits apoptosis in various cells lines, suggesting that nicotine has the ability not only to promote lung cancer development by activating cell growth pathways, but also to reduce the efficacy of chemotherapeutic agents by stimulating survival pathways.[48–50] It has been postulated that nicotine-induced resistance to proapoptotic chemotherapy occurs through modulation of mitochondrial signaling[51,52] Nicotine prevented chemotherapy-induced reduction of mitochondrial membrane potential, activation of caspase-9, and translocation of Bax to the mitochondria. In addition, AKT-mediated phosphorylation of proapoptotic protein Bad, and upregulation of antiapoptotic protein, XIAP, was observed in cells exposed to nicotine.[51,53] Inhibition of mitogen-activated protein kinase and AKT prevents the antiapoptotic effects of nicotine and decreased chemotherapy-induced apoptosis. Others have demonstrated that combined exposure to nicotine and cigarette smoke carcinogen, nicotine-derived nitrosamine ketone, for a week augmented *Bcl-2* expression and increased resistance to cisplatin-mediated apoptosis.[54] More recently, it has also been shown that other than these direct effects, nicotine and its derivatives can stimulate the release of stress hormones from cancer cells, leading to increased cancer cell proliferation.[55]

There are no human studies directly studying the effects of nicotine on chemotherapy effects or toxicities. A detailed discussion on molecular effects of nicotine as relates to lung cancer signaling pathways is beyond the scope of this article and the reader is referred to recent reviews that provide comprehensive analysis of the literature.[50,56,57] We will limit this review to direct effects of smoking on metabolism of often used systemic therapy for lung cancer.

EFFECT OF SMOKING ON SYSTEMIC THERAPY FOR LUNG CANCER

The literature investigating the effect of tobacco smoke on the catabolism of commonly used chemotherapeutic agents is relatively limited, especially drugs used to treat lung cancer. Given the known association of smoking with lung cancer, a review of this interaction and effect is both necessary and clinically relevant. Moreover, although this interaction has been investigated with a small number of chemotherapeutic agents, the effects of drug efficacy and outcome have not yet been clearly delineated for many drugs. A more clear understanding of mechanism, effects, and outcome may allow clinicians to tailor chemotherapy drug choice according to known factors, including whether the patient is a current, former, or never smoker.

ERLOTINIB

Erlotinib is an orally active potent selective inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase. Erlotinib inhibits EGFR-dependent proliferation of tumor cells in vitro and blocks cell cycle progression in the G1 phase.[58,59] Erlotinib is predominately metabolized by CYP3A4 and a lesser extent by CYP1A2 and CYP1A1 enzymes involving demethylation of side chains and oxidation to carboxylic acid metabolites.[60] Cigarette smoking can accelerate catabolism and result in lower plasma concentrations of erlotinib.

Studies have demonstrated variability in survival for lung cancer patients receiving erlotinib based on smoking status. In a retrospective analysis of 88 patients with advanced non–small-cell lung cancer (NSCLC) who received erlotinib or pemetrexed as second-line therapy, erlotinib achieved significantly better progression-free survival (PFS) in never smokers when compared with former smokers (3.5 versus 2.7 months, $p = 0.005$).[61] Similarly, never smokers with squamous histology receiving erlotinib lived longer compared with ever smokers.[62] Moreover, in a comparative analysis of the efficacy of second-line docetaxel and erlotinib treatment in patients with advanced NSCLC, heavy smoking history in erlotinib-treated patients was associated with decreased overall survival (hazard ration [HR] 3.61 [1.77–7.4], $p = 0.0005$).[63] In the phase III trial by Shepherd et al.[64] entitled BR.21, smoking history was the only clinically relevant factor showing interaction with erlotinib treatment, indicating that erlotinib was more effective in patients who had never smoked, when compared with patients who were either current or former smokers. Specifically, 24.7% of never smokers had complete or partial responses to therapy versus 3.9% of current or ever smokers ($p < 0.001$). The median survival for patients on erlotinib that never smoked was 12.3 months (HR 0.42), former smokers 5.5 months (HR 0.84), and current smokers 6.1 months (HR 0.93) ($p < 0.006$).[64–66] It is true that many nonsmoking related lung adenocarcinoma that respond to EGFR-targeted tyrosine kinase inhibitors have an activating mutation in the *EGFR* gene. However, pharmacokinetically, current smokers had nearly a twofold reduction in measured trough erlotinib plasma levels compared with former and never smokers at 24 hours postdose,[65] which suggests that factors other than EGFR mutation status may result in altered drug response. Lu et al.[67] analyzed data from seven erlotinib clinical trials in which erlotinib was administered at 150 mg once daily; the primary objective of the study was to characterize the PK of erlotinib in patients with solid tumors and identify factors that had an impact on PK. PK data were available for 1047 of 1859 (56%) of patients randomized to treatment with erlotinib in the seven trials evaluated. Current smokers had median steady-state erlotinib trough plasma concentrations (C_{24h}) approximately half that of patients who were former smokers. Furthermore, smoking status was found to be a significant covariate affecting drug clearance. Current smokers had a 23.5% increase in clearance compared with never and former smokers. Thus, the result of smoking effect on clearance was consistent with in vitro data that demonstrated induction of CYP enzymes by cigarette smoke.[12]

To better evaluate the question whether the decreased erlotinib exposure seen in current smoking cancer patients versus former or never smokers was because of their smoking status, Hamilton et al.[68] designed a phase I, single-center, open-label PK crossover study. The study enrolled 32 male subjects, both nonsmokers (defined as subjects who had not consumed tobacco or nicotine-containing products for 1 year before the start of the study) and current smokers (defined as having smoked a minimum of 10 cigarettes per day for greater than or equal to 1 year and have a positive test for cotinine). All subjects received a single dose of 150 mg erlotinib on day 1 followed by a single dose of 300 mg erlotinib on day 15. Eleven plasma sampling times were used to determine the PK profile for each subject, and PKs of each cohort were compared. Results showed that smokers experienced decreased exposure to erlotinib when compared with nonsmokers; both the analytes of erlotinib and OSI-420 (the main metabolite of the drug) were eliminated more rapidly in smokers than nonsmokers, and the dose to reach an area under the concentration curve (AUC) in the therapeutic range was 300 mg for smokers versus 150 mg for nonsmokers. The ratio of AUC for smokers to nonsmokers was 35.9% ($p < 0.0001$) and ratio of 24-hour concentration was 12.1% ($p = 0.0001$). In addition, this study found that the C_{max} of smokers was approximately two-third of that of nonsmokers and the C_{24h} of smokers was 8.3-fold lower than that of nonsmokers. These findings confirmed that smoking does alter the PK of erlotinib with decreased plasma concentrations and increased clearance in smokers. It was hypothesized that these findings may be due, in part, to induction of the cytochrome P450 CYP1A1 and CYP1A2 isoforms by cigarette smoke, leading to increased catabolism and clearance. The clinical implications are also important to note that nonsmokers had a higher incidence of adverse events, including rash and diarrhea, than current smokers, possibly indicating increased erlotinib exposure compared with the smoker cohort. Conversely, studies have also shown that smokers receiving erlotinib have increased *CYP1A1* mRNA in the lungs, which then generate reactive quinone-imine and epoxide intermediates, exhausting cellular glutathione stores, and leading to increased interstitial lung disease.[69] Li et al.[69] showed that smokers receiving another EGFR inhibitor gefitinib had a 12-fold increase in glutathione adduct formation compared with nonsmokers, consistent with an upregulation of CYP1A1 in pulmonary microsomes (Table 1). Similarities

TABLE 1. Potential Pharmacokinetic and Pharmacodynamic Effects of Smoking-induced Induction of Drug-Metabolizing Enzymes in Often Used Systemic Therapy in Lung Cancer

Drug	Area Under Curve	C_{max}	CL	Enzymes	Drug Target	Reactive Intermediates	Adverse Reactions
Erlotinib	↓	↓	↑	CYP3A4, CYP1A2, and CYP1A1	HER1/EGFR	Quinone-imines epoxides	↓Rash, diarrhea ↑DILI*, ILD†, SJS
Docetaxel	NC	NC	NC	CYP3A4	Antimitotic	—	↓Neutropenia, anemia, thrombocytopenia, alopecia
Paclitaxel	NC	NC	NC	CYP3A4 and CYP2C8	Antimitotic	—	↓Neutropenia, anemia, thrombocytopenia, alopecia
Irinotecan	↓	↓	↑	UGT1A1 and CYP3A	Topoisomerase-I inhibitor	—	↓Neutropenia, leukopenia, diarrhea
Gemcitabine	U	U	U	Cytidine deaminase	Cytidine analogue	—	↓Neutropenia
Gefitinib	↓	↓	↑	CYP3A4, CYP1A2, and CYP1A1	HER1/EGFR	Quinone-imines epoxides	↓Rash, diarrhea ↑DILI, ILD‡

Increase in parameter in represented by ↑ and decrease by ↓.
*DILI, drug-induced liver injury.
†ILD (interstitial lung disease), increased ILD because of increased *CYP1A1* mRNA in lungs generating the reactive intermediate, exhausting cellular glutathione stores.[69]
‡ILD (interstitial lung disease), 12-fold increase in glutathione adduct formation in smokers compared with nonsmokers. This increase is consistent with an upregulation of CYP1A1 in pulmonary microsomes from smokers.[9]
CL, clearance; NC, no change; SJS, Stevens-Johnson syndrome; U, unknown.

between gefitinib and erlotinib metabolism has been reviewed by Duckett and Cameron.[70] Based on PK findings and clinical implications, the possibility of dose escalation of erlotinib remains a viable option.

A recent study determined maximum-tolerated dose (MTD) in smokers and compared PK profiles with standard dosing.[71] In the first part of the study, a standard dose escalation study was performed in a cohort of patients receiving 200–350 mg of erlotinib per day, escalating in 50 mg increments. Patients were monitored for dose-limiting toxicities while taking the drug for 14 days. The Phase I component of the study determined that the MTD for smokers was 300 mg per day. In the Phase II portion, the PK and toxicity profile for smokers receiving 300 mg daily was found to be similar to nonsmokers receiving 150 mg daily, which confirmed that current smokers both have increased clearance and tolerate dose escalation. Based on these findings, this study concluded that the recommended Phase II dose of erlotinib was 300 mg for current smokers. Moreover, an exploratory survival analysis also noted a trend towards longer median survival for smokers receiving the escalated dose of erlotinib.

Additional prospective studies are required to determine whether an increased dose of erlotinib in smokers without an activating mutation of EGFR affects survival outcomes. It is also unclear whether EGFR-wild type patients may be targeted by increasing the half maximal inhibitory concentration (IC_{50}) of EGFR inhibitors and such studies are also underway.[72] A prospective study by Petty et al.[59] revealed that smoking status was associated with a higher MTD of erlotinib; however, this increased dose did not favorably affect PFS. In summary, the use of erlotinib in advanced lung adenocarcinoma is currently approved for patients with an activating EGFR mutation. The recently completed ERMETIC study demonstrated that patients with identified EGFR mutation had better outcomes with erlotinib.[73] However, there is ample data that a small subset of patients with wild-type *EGFR* genes may also respond to erlotinib, albeit to a lesser extent.[74,75] Although the magnitude of PFS improvement was not as great as those patients with EGFR mutations, for this group of patients, one could consider differential dosing of erlotinib based on smoking status. The most crucial intervention is smoking cessation. However, consideration may be given to altered PKs of erlotinib in patients who have recently quit smoking, yet still demonstate increased cytochrome P450 isoforms.

TAXANES

Taxanes are used as a first-line treatment of NSCLC, often with a platinum compound. Both docetaxel and paclitaxel are antimicrotubule agents metabolized by CYP3A and CYP2C8.[76,77] Prior studies have suggested that the PKs of paclitaxel are greatly influenced by external factors, including environmental and genetic factors. To determine the effects of smoking on the PK and toxicity profiles of both docetaxel and paclitaxel tumors, de Graan et al.[78] conducted a retrospective study of 566 patients with solid tumors enrolled in multiple studies conducting PK analyses. Docetaxel-treated patients received 75–100 mg/m^2 dose, and paclitaxel-treated patients received 90 mg/m^2 weekly or 175 mg/m^2 every 3 weeks. Upon analysis of PK parameters (calculated by nonlinear mixed effect modeling population analysis), cigarette smoking was not found to alter the PK determinants of either drug, but smokers treated with docetaxel and paclitaxel were found to have lower incidence of neutropenia and leukopenia. Specifically, smokers treated with docetaxel showed less grade 4 neutropenia (35% versus 52%; $p = 0.01$) than nonsmokers. Smokers treated with paclitaxel had less grade 3-4 leukopenia than nonsmokers (12% versus 35%; $p = 0.03$) and the white blood cells nadir was lower in nonsmokers (median 2.7×10^9/L; range $0.05–11.6 \times 10^9$/L) than in smokers (median 3.3×10^9/L; range $0.8–10.2 \times 10^9$/L; $p = 0.02$). The study investigators concluded that further research was warranted to clarify the underlying mechanisms of this potential protective effect of smoking on hematologic toxicities in taxane therapy. Other studies have shown that it is the time above paclitaxel plasma

concentration of 0.05 to 0.2 μmol/l ($t_{c>0.05–0.2}$) is a better predictor of treatment-related neutropenia than clearance.[79] In summary, smokers treated with taxanes seem to have less grade 3/4 severe neutropenia. The exact reasons for this are unclear; further studies are needed to determine whether a lesser degree of neutropenia is a pharmacodynamic (PD) predictor of poor response to taxanes in smokers with advanced NSCLC.

IRINOTECAN

The constituents of tobacco smoke have similarly been found to alter the PK and adverse effects of irinotecan (CPT-11), a topoisomerase-I inhibitor that is approved for treatment of small-cell lung cancer. Irinotecan is a known substrate for several cytochrome P450 and UGT1A1 isoenzymes, which are known to be induced by tobacco smoke. In addition, in phase I studies, most responses to irinotecan were observed at the highest dose levels, indicating a clear-dose response relationship with this drug.[80] In vitro studies have demonstrated that smoking may affect the partitioning of irinotecan in red blood cells (RBCs).[81] Dumez et al.[81] performed in vitro incubations of blood from both smoker and nonsmoker volunteers with irinotecan over a concentration gradient to investigate the changes in partitioning between RBCs. After extraction and sample pretreatment, high-performance liquid chromatography and fluorescence detection were used to determine drug concentration in the different blood consitutents. Although irinotecan generally showed relatively high affinity for the RBCs, there was a higher concentration of irinotecan in the erythrocytes of nonsmokers when compared with smokers. It was theorized that constituents of tobacco smoke, such as arylamines, directly prevented RBCs from interacting with irinotecan. Because RBCs play a key role in drug transport because of their long lifetime, higher drug concentrations in nonsmokers versus smokers may have clinical implications.

To better investigate the potential clinical effect of smoking on irinotecan PKs, van der Bol et al.[82] reviewed the data of 202 patients who received irinotecan through nine prospective trials between 1996 and 2005. Patients received irinotecan once every 3 weeks as a 90-minute intravenous infusion at doses ranging from 175 to 350 mg/m^2 or a 600 mg flat dose. Blood samples were collected at set time points up to 500 hours after the infusion for measurements of irinotecan and its metabolites, SN-38 and SN-38G. Samples were analyzed by high-performance liquid chromatography. A total of 190 patients (49 smokers and 141 nonsmokers) were assessable to evaluate the effect of smoking on the PK properties of irinotecan. The dose-normalized area under the plasma concentration-time curve of irinotecan was significantly lower in smokers than in nonsmokers. Smokers also showed an 18% faster clearance of irinotecan than nonsmokers (median, 34.8 versus 29.5 l/hour, $p = 0.001$) and 40% lower systemic exposure to the active irinotecan metabolite SN-38 when compared with never smokers. Notably, a specific exclusion criterion for the analysis was the use of known CYP3A or UGT1A1 inducers or inhibitors, thereby reducing potential confounding metabolic phenotypes. The analysis also revealed that smokers who received irinotecan were found to experience significantly less hematologic toxicity, including considerably less neutropenia. Specifically, the median white blood cell values decreased to 5.3×10^9/l in smokers and 3.0×10^9/l in nonsmokers ($p < 0.001$); the respective absolute neutrophil count values were 3.3×10^9/l versus 1.6×10^9/l ($p < 0.001$). The authors concluded that smoking significantly affects the PK and toxicity profile of irinotecan. It was posited that the more extensive glucuronidation of SN-38 in smokers resulted in reduced systemic exposure to the active metabolite, thereby likely contributing to less hematologic toxicity. Although the exact underlying mechanism remains to be elucidated, the effects of smoking on irinotecan PK may be ascribed to induction and modulation of CYP3A and UGT1A1 enzymes, which are involved in irinotecan metabolism. In summary, reduced exposure to irinotecan is seen in current smokers. However, it is unclear whether this affects responsiveness of lung cancer to irinotecan.

GEMCITABINE

Gemcitabine is a prodrug, a deoxycytidine analogue that requires intracellular uptake and phosphorylation to be activated. The drug is phosphorylated to gemcitabine monophosphate (dFdCMP) by deoxycytidine kinase, which is then converted to the active metabolites, gemcitabine di- and triphosphate nucleosides (dFdCDP and dFdCTP). This nucleoside analogue exhibit cytotoxic effects through inhibition of DNA synthesis. Gemcitabine is inactivated by cytidine deaminase (CDA) mediated conversion to the inactive metabolite, difluorodeoxyuridine (dFdU).[83]

Gemcitabine has been studied extensively as both a single agent and in combination with a platinum-based compound in both first and second-line setting for lung cancer.[84–87] In these studies, doses ranged from 800 to 1200 mg/m^2 weekly administered for 3–4 weeks. Gemcitabine is fairly well tolerated, with the principal hematologic toxicity being grade 3/4 neutropenia in 10–20% of patients. The overall response rate in nearly 600 assessable patients was 21%.[88] The recommended dosage regimen for gemcitabine monotherapy is 1000 mg/m^2 doses administered over 30 minutes on days 1, 8, and 15. CDA is a key enzyme in gemcitabine metabolism. Previous studies have shown SNPs in the *CDA* gene that may affect the PK and toxicity of gemcitabine.[89] However, the indentified SNPs occur in low allele frequencies and are unlikely to explain the entire story.[90] Although little is known regarding the effect of smoking on its PK in vivo, smoking has been shown to increase the expression of *CDA*.[91] This overexpression may lead to increased catabolism and reduced efficacy of the drug in smokers and former smokers.

Kanai et al.[92] performed a retrospective review of 103 patients receiving gemcitabine monotherapy at Kyoto University Hospital over a 4-year period and obtained information regarding smoking history and incidence of grade 3–4 gemcitabine-induced neutropenia from the medical record. Gemcitabine was initiated at doses of 460–1000 mg/m^2 over 30 minutes on days 1, 8, and 15 of a 28-day cycle. Logistic regression analysis was carried to out to investigate the correlation between gemcitabine-induced neutropenia and various clinical factors, including smoking history. Of the 103 patients, 51 were smokers (current and former smokers) and 52 nonsmokers. Overall, smokers were found to have a lower incidence of grade 3–4 neutropenia as nonsmokers (24% vs. 56%; odds

ratio 0.244, 95% confidence interval 0.105–0.569; $p < 0.001$). After logistic regression analysis, smoking emerged as an independent inverse predictor of gemcitabine-induced neutropenia (odds ratio 0.188, 95% confidence interval 0.057–0.618; $p = 0.006$). In addition, dose intensity of gemcitabine was significantly lower in the nonsmoker group than in the smoker group (0.59 mg/m^2 versus 0.68 mg/m^2; $p = 0.03$), indicating that dose reduction was more common in nonsmokers versus smokers, presumably necessitated by treatment-related neutropenia. The exact mechanism behind this inverse correlation identified by Kanai et al.[92] is unclear. In our retrospective study of gemcitabine in 151 patients, albeit in mixed solid tumors, smokers had less neutropenia than nonsmokers, a finding that was more pronounced with increasing pack-years.[48] A steep dose-response curve for gemcitabine is not seen in lung cancer as in many other solid tumors. However, there is both preclinical and clinical data to support the premise that higher doses are associated with greater responses. In a preclinical study by Von Hoff,[93] a concentration of 22 mcg was associated with higher clonogenic cell kill compared with 2 mcg. In a phase I study of the 29 patients receiving gemcitabine weekly, treatment 3 weeks on and 1 week off only seven patients had a clinical response (six at the 2200 mg/m^2 dose level and one at the 2800 mg/m^2 dose level).[94]

In summary, smokers receiving gemcitabine monotherapy for lung cancer have a lower incidence of grade 3/4 neutropenia compared with nonsmokers; however, the mechanism remains unclear. Whether this PD end point is associated with clinically meaningful difference in response to gemcitabine in humans requires further investigation.

PLATINUM-BASED REGIMENS

Components in smoke can block induced apoptosis or promote cellular proliferation[54] limiting the efficacy of key first-line lung cancer agents, such as cisplatin or carboplatin. It is generally accepted that DNA damage and subsequent induction of apoptosis may be the primary cytotoxic mechanism of platinum compounds. They are used as first-line chemotherapy for lung cancers. These drugs are primarily excreted renally unchanged, thus suffer no PK alterations by compounds in smoke; however, the cellular response to platinum-based agents can be altered by smoking status. As mentioned above, nicotine can block activation of apoptotic pathways such as caspase-9, translocation of Bax or phosphorylation of Bad and Bcl-2 in cell lines. Other compounds, such as NNKs, can reduce expression of proapoptoic factors such as Bcl2.[56] Nicotine or nicotine-derived nitrosamine ketone can antagonize the therapeutic efficacy of cisplatin and carboplatin regimens by directly blocking apoptosis.

In addition, cigarette smoke carcinogens have been shown to alter expression of key genes involved in platinum-induced apoptosis through DNA methylation. Studies have shown that the expression of DNA methylating enzymes (DNA methyltransferases) can be modified in response to cigarette smoking.[95] In particular, micro RNA, mIR-143, that regulates DNTMT3A has been shown to be downregulated in NSCLC and associated with smoking status.[96] Smoking-induced alterations to DNA methylation of key apoptotic genes can limit the efficacy of cisplatin and carboplatin. Consider the human high temperature requirement factor A3 (HtrA3), a mitochondrial stressed-induced serine protease, which has been described as a key factor in modulating sensitivity to cisplatin in smoking-related lung cancer.[97] NNKs can regulate HtrA3 expression through exon 1 methylation. Beleford et al.[97] demonstrated that HtrA3 expression is reduced or absent in over 50% of lung cancer cell lines and primary lung tumors from heavy smokers. Treatment of HtrA3-deficient cell lines with 5-aza-2'-deoxycytidine, a DNA methyltransferase inhibitor, resulted in a dose-dependent increase in HtrA3 transcription. Further sequence analysis of bisulfite-modified DNA from lung cancer cell lines and primary lung tumors showed an increased frequency of methylation within the first exon of HtrA3 with a corresponding loss of expression, particularly in tumors from smokers. Resistance to cisplatin cytotoxicity was a functional consequence of HtrA3 loss because of altered methylation.[98] The study also demonstrated similar findings for the topoisomerase inhibitor, etoposide. There are currently no human studies demonstrating the clinical relevance of these epigenetic effects of smoking on platinum activity.

EFFECTS OF NICOTINE AND NICOTINE REPLACEMENT THERAPIES ON METABOLISM OF SYSTEMIC THERAPIES FOR LUNG CANCER

Given the increase in prescriptions for various forms of nicotine replacement therapies, we looked for studies addressing any PK interactions between these and systemic chemotherapy for lung cancer. Nicotine is extensively metabolized in the liver by several enzymes including the cytochrome eP450 system (CYP2A6), aldehyde oxidases and uridine 5′-diphosphate-glucuronyl transferases. Various drugs that use these metabolic pathways can have an effect on nicotine metabolism as reviewed by Hukkanen et al.[99] However, there are no human trials evaluating PK interactions between nicotine replacement therapies and systemic therapies used to treat lung cancer. Varenicline, a partial agonist at nicotinic acetylcholine receptors, is widely used and a successful smoking cessation aid. Preclinical studies showed a high affinity of varenicline for 5HT3 receptors.[100] 5-HT$_3$ receptor antagonists are in use clinically, primarily for controlling chemotherapy- and radiotherapy-induced nausea and vomiting. However, it is unclear whether this results in clinically relevant drug interactions. There are no reported studies of interactions between varenicline and often used systemic therapies for lung cancer

CONCLUSION AND SUMMARY

Cigarette smoking is an independent factor relating to poor outcomes after a diagnosis of lung cancer. Herein, we reviewed the literature for the effects of smoking on often used systemic therapy for lung cancer. Admittedly, there are multiple biological reasons for variable PK/PD effects related to smoking and systemic cancer therapy effects. Results from preclinical experiments have not been rigorously studied in clinical settings. We primarily addressed potential differences in drug metabolism in smokers versus nonsmokers. Other factors include inherent biological differences in smoking-related cancers that are well described, such as presence of specific

mutations (e.g., Kras), decreased apoptotic potential, or highly complex heterogeneous tumors that do not lend themselves to effective cell kill from current therapies. Host factors in smokers may include a complex interplay with polymorphisms in common metabolizing enzymes, and impairments in absorption, distribution, and excretion of specific systemic therapies. These limitations notwithstanding, it is apparent that smoking leads to accelerated clearance of specific systemic therapies.

The question arises whether an increase in dose of systemic therapies for lung cancer will result in a clinically significant difference in response or overall outcomes. There is a paucity of data specifically addressing a dose-response effect of lung cancer specific systemic therapies in smokers. There are no prospective studies that specifically link smoking-mediated induction of drug metabolism to therapy efficacy. Although this phenomenon is fairly well described in the literature for non-chemotherapy medications, the data investigating the effect on chemotherapy drugs are limited and primarily restricted to retrospective data and PK studies. It can be posited that reduced concentrations of systemic therapy related to increased catabolism may lead to reduced efficacy of the drug in former smokers. Therefore, smokers (including smokers who recently quit) may then require a higher therapy dose overcome the likelihood of accelerated catabolism. PK parameters should be studied, particularly in former smokers. Whether individualized chemotherapy dose algorithms according to metabolic phenotype and smoking history will impact patient outcomes remains to be seen. Thus, prospective trials comparing chemotherapy PKs in smokers versus nonsmokers are required. Similarly, although there is preclinical data to suggest that nicotine itself is proliferative and antiapoptotic in cell culture systems, prospective trials are needed to determine whether nicotine (including replacement cessation therapies) can lead to interactions with often used systemic therapies for lung cancer which may limit therapeutic efficacy.

In summary, PK and PD interactions between cigarette smoking and chemotherapy drugs may significantly impact drug clearance, delivery, toxicity, and efficacy. Cigarette smoking history must be carefully considered as a patient-specific factor capable of impacting treatment and potentially disease outcome. Smoking cessation must be emphasized at every clinical encounter.[101,102] However, in former smokers, dysregulation of drug-metabolizing enzymes may persist and potentially leading to increased clearance. These are the patients who will benefit from additional well-conducted clinical trials. Findings could yield potential therapeutic targets that could be modulated according to histone acetylation or DNA methylation status, particularly if epigenetic effects are responsible for keeping these metabolizing enzymes in a persistently activated state. Optimally, clinical trials should take this factor into consideration and potentially use smoking status as an independent predictive variable when designing studies of personalized medicine.

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